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THE EFFECT OF TEMPERATURE AND LIGHT ON THE DEVELOPMENT OF THE UREDINIAL STAGE OF *PUCCINIA GRAMINIS* PERS.

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BY

LEONARD WILLIAM MELANDER

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EFFECT OF TEMPERATURE AND LIGHT ON DEVELOPMENT OF THE UREDIAL STAGE OF PUCCINIA GRAMINIS

BY

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(Contribution from Bureau of Plant Industry)

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EFFECT OF TEMPERATURE AND LIGHT ON DEVELOPMENT OF THE UREDIAL STAGE OF PUCCINIA GRAMINIS[1]

By LEONARD W. MELANDER[2]

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INTRODUCTION

One of the important problems in the study of the epidemiology of stem rust, *Puccinia graminis* Pers., concerns the extent to which the uredial stage can persist through the winter under different conditions. The ability of this stage to survive obviously depends upon the ability of the urediospores or of the uredial mycelium in grains and grasses to withstand fluctuating temperatures and other conditions during the winter. It seems clear from the investigations of Stakman et al., as cited by Lambert (*12*),[3] that *P. graminis* does not commonly overwinter in the uredial stage north of Texas. As temperature, moisture, and light are likely to be among the most important physical factors involved, experiments were made to determine their effect.

HISTORICAL REVIEW

In the northern part of the United States the fate of the uredial stage of *Puccinia graminis* in the winter has been studied many years. In March 1905 Bolley and Pritchard (*4*) collected viable urediospores from leaves of quackgrass and wild barley frozen in the ice at Fargo, N. Dak. In 1906–07 Freeman and Johnson (*6*) were able to find viable urediospores on *Hordeum jubatum* L. and *Agropyron repens* (L.) Beauv. as late as April 15. In 1911 Hungerford (*10*) showed that urediospores of timothy stem rust, *P. graminis phlei-pratensis* (Eriks. and Henn.) Stak. and Piem., persisted through the winter in Wisconsin, while Stakman and Piemeisel (*24*) observed that they "survived the very severe winter of 1916–17 at St. Paul, Minn., very easily." It is evident, however, that *P. graminis phlei-pratensis* differs from other varieties of *P. graminis* in that its uredial stage can survive the winter in the northern United States more easily

[1] Received for publication Feb. 12, 1935; issued July 1935. Cooperative investigations between the Division of Cereal Crops and Diseases and the former Division of Barberry Eradication, Bureau of Plant Industry, U. S. Department of Agriculture, and the Agricultural Experiment Station of the University of Minnesota. Presented to the faculty of the Graduate School of the University of Minnesota as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy granted in June 1930.

[2] Now in the Division of Plant Disease Eradication and Control, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. Acknowledgement is made to Dr. E. C. Stakman, University of Minnesota, for suggesting the problem and for helpful criticism during its prosecution; to Dr. R. B. Harvey, University of Minnesota, for suggestions relative to some of the physiological phases; and to Dr. M. N. Levine, Division of Cereal Crops and Diseases, for criticism and suggestions. The writer is also indebted to Dr. Stakman and his coworkers for the cultures of *Puccinia graminis* and other material and to H. G. Ukkelberg, University Farm, St. Paul, Minn., for assistance in some of the routine work.

[3] Reference is made by number (italic) to Literature Cited, p. 879.

than can that of other varieties. Lambert (*12, p. 27*) has summarized some of the extensive studies of Stakman et al. made—

> throughout the Mississippi Valley to determine the northern limits of overwintering of *P. graminis tritici* in the uredinial stage. * * * In general, they indicate that, north of Oklahoma, primary infection of cereals as a result of local overwintering in the uredinial stage, occurs so rarely that it is probably of no practical importance.

Hungerford (*11*) could not confirm the theory of Pritchard (*19, 20*) that the mycelium of *P. graminis* overwintered in seed wheat as mycelium and that when the seed germinated the mycelium grew by penetrating "various parts of the seedling, invading both the intercellular spaces and the cells."

It seems clear, therefore, that the uredial stage of most of the varieties of *Puccinia graminis* investigated does not commonly survive the winter in the northern United States. The question then arises whether urediospores and the uredial mycelium in perennial grasses and winter grains are killed by the cold or by alternate freezing and thawing. In an attempt to answer this question experiments were made to determine the resistance of urediospores and mycelium to low temperature as well as to fluctuating temperature. It is possible that the ability of urediospores to withstand extremely low temperature may depend somewhat upon the temperature and light conditions that prevailed prior to freezing. Experiments also were made, therefore, to determine the effect of temperature and light on the development of the uredial stage.

MATERIALS AND METHODS

Experiments on the effect of temperature and light were made in four large chambers kept at constant temperatures regulated by a large, automatically controlled refrigerating machine. The chambers were approximately 10 feet square and were lighted artificially by 1,000-watt incandescent lamps. To maintain uniform temperature and humidity, electric fans were kept running continuously. In addition to the four temperature chambers, an ice box cooled by a special refrigerating machine was used for extremely low temperatures. The greenhouse in which the plants were incubated in moist chambers was kept at about 20° C. by means of automatic heat controls.

Temperatures were recorded every 10 minutes by a Leeds-Northrop electrical recording machine and deviations from the desired temperature were corrected immediately, although the variation usually was not more than +1° C.

Several physiologic forms of *Puccinia graminis tritici* Eriks. and Henn., *P. graminis avenae* Eriks. and Henn., and *P. graminis secalis* Eriks. and Henn., and one collection each of *P. graminis phlei-pratensis* and *P. triticina* Eriks. were used. The histories of these forms are shown in table 1. The forms studied, except those of *P. graminis phlei-pratensis* and *P. triticina*, were cultured on some or all of the differentials of wheat, oats, and rye used in identifying the forms (*1, 5, 23*). The spore measurements were made under the 4-mm objective by means of a Zeiss ocular screw micrometer attached to a Spencer microscope.

Urediospores were hardened by placing plants heavily infected with the uredial stage in the 0° C. room for at least 10 days. This process was the same as that used by Harvey (*9*) for hardening higher plants before subjecting them to subzero temperatures.

TABLE 1.—*History of varieties and physiologic forms of Puccinia graminis and P. triticina used in studies of the effect of temperature and light on the uredial stage*

Species, variety, and form	Original host	Date collected	Locality in which collected	Collector
P. graminis tritici:				
Form 15	Wheat	May 12, 1923	Pusa, India	R. R. Sen.
Form 35	do	Sept. 2, 1926	St. Paul, Minn	E. Miller.
Form 36	do	Aug. 22, 1924	Morris, Minn	M. N. Levine.
P. graminis avenae:				
Form 5	Oats	Aug. 13, 1929	Morrisville, N. Y	Do.
Form 2	do	Aug. 17, 1929	Mankato, Minn	R. H. Bamberg, Lee Person.
P. graminis secalis:				
Form 7 (brown)	*Agropyron repens*	Aug. 9, 1927	St. Peter, Minn	C. L. Lefebvre et al.
Form 7 (orange)[1]	Rye	Mar. 3, 1929	Greenhouse, University Farm, St. Paul, Minn.	L. W. Melander.
P. graminis phlei-pratensis.	Timothy	Sept. 25, 1929	Scott County, Minn	Do.
P. triticina	Wheat	1930	St. Paul, Minn.[2]	

[1] Originated apparently as a mutation from the brown culture of *Puccinia graminis secalis* form 7; according to Cotter and Levine (*5*), there was no difference in the pathogenicity of the two strains.
[2] Contamination in greenhouse.

With few exceptions, the method of inoculation and incubation was uniform and similar to the one described by Cotter and Levine (*5*). Seedling plants first were sprayed with water and then inoculated by brushing them with rusted plants. The inoculated plants were placed in incubation chambers, again sprayed with water, kept at a temperature of 20° C. for 48 hours, and then placed under the conditions provided for a given experiment. All plants were watched closely for the first appearance of flecks and uredia. When the uredia had reached their maximum development, notes were taken on the number of plants inoculated and infected and on the type of infection produced.

Several experiments failed in the low light intensities because root rot killed the plants before rust pustules appeared. Thereafter all seed was treated with Ceresan [4] to delay damage from root rot until after uredia had developed and final notes had been taken.

RESULTS

EFFECT OF TEMPERATURE

In general, a temperature of 20° C. seems most favorable for the development of the uredial stage of *Puccinia graminis*, while subzero centigrade temperatures may kill urediospores, especially when moist, and temperatures just above zero may retard development by lengthening the incubation period and by preventing growth and sporulation. The effect of temperature may vary with varieties and physiologic forms. An account of the results of controlled experiments on these phases of the problem follows.

EFFECT OF CONSTANT SUBZERO CENTIGRADE TEMPERATURES ON VIABILITY OF UREDIOSPORES

The effect of outdoor conditions in winter on the viability of urediospores has been studied by various investigators (*3*, *4*, *6*, *17*, *18*, *20*),

[4] An organic mercury compound used in the treatment of seed for the prevention of numerous seed-borne diseases.

but very little is known concerning the effect of temperatures below 0° C. exclusive of other factors. Mehta (*16*) found that—

* * * after exposure to −6.5° to −10° C. for 24 hours, the urediniospores of this rust (*Puccinia graminis*) do not show even 10 percent germination and after four to seven days there is a total loss (only one or two spores germinating) of viability.

In the present work urediospores were exposed to temperatures of −29° to −40° C. constantly for various periods. The question arose whether hardening had any effect on the viability of urediospores. This is important because, in the Northern States, urediospores are exposed in the fall to temperatures just above 0° before the onset of cold winter weather. That fungi can be hardened has been demonstrated. Bartetzko (*2*), in 1909, showed that the minimum temperatures withstood by *Aspergillus niger* Van Tieg. could be lowered by first hardening, or exposing the fungus to a temperature just above freezing.

Numerous investigators (*4*, *5*, *6*) have observed that urediospores of *Puccinia triticina* live over the winter in the northern United States. In view of this fact, urediospores of this rust were frozen along with those of *P. graminis tritici*, *P. graminis avenae*, and *P. graminis phlei-pratensis*.

Spores of approximately the same age were dusted on dry glass slides in individual small pasteboard boxes. These were placed in an ice box maintained at temperatures below −29° C. Germination tests were made at the end of 1, 2, 3, 5, 10, 15, 20, 25, 30, 35, 40, and 45 days. Hardened spores were obtained from plants kept at 0° to 1° for at least 10 days after the uredia were mature, and nonhardened spores from plants inoculated at the same time but kept continuously in the greenhouse. After exposure to low temperatures, the spores to be tested were placed in a drop of distilled water on a glass slide and kept at 20° for 24 hours, after which counts were made.

That some urediospores of the *tritici* and *phlei-pratensis* varieties of *Puccinia graminis* can survive temperatures ranging from −29° to −40° C. for at least 45 days is shown in table 2. It appears that because of the sudden change in temperature more spores are killed during the first 24 hours than during any subsequent period. The viability of hardened spores of *P. graminis tritici* (table 2) was least affected by low temperature, and nonhardened spores of *P. graminis avenae* form 2 succumbed most readily, being unable to withstand the low temperature for more than 20 days in 2 out of 3 trials.

Hardened spores of *Puccinia graminis tritici* withstood temperatures of −29° to −40° C. better than nonhardened spores (table 2 and fig. 1). While very few spores of *P. graminis avenae* survived, hardening seemed to enable some of them to survive prolonged exposure to −29° to −40°. There seemed to be little or no difference in ability of hardened and nonhardened urediospores of *P. triticina* to withstand low temperature.

Clearly, some urediospores of *Puccinia graminis*, when kept dry, can withstand considerable exposure to extremely low temperatures. This probably is true under natural conditions also, where long periods of continuous subzero centigrade temperature often prevail. The rather low temperatures of late fall are sufficient to harden urediospores and enable more of them to withstand constant subzero temperatures for long periods, but the question arises as to the effect on

the viability of urediospores of temperatures alternating above and below 0° C.

TABLE 2.—*Effect of hardening on viability of urediospores of Puccinia graminis and P. triticina at −29° to −40° C.*

Days at −29° to −40° C. (number)	Percent germination [1] of urediospores of—							
	P. graminis tritici form 15		*P. graminis avenae* form 2		*P. graminis phlei-pratensis*		*P. triticina*	
	Hardened	Nonhardened	Hardened	Nonhardened	Hardened	Nonhardened	Hardened	Nonhardened
0	41.3	55.2	17.6	33.5	45.7	28.5	17.1	40.1
1	6.1	3.0	6.9	2.0	5.7	1.6	10.2	2.7
2	6.4	6.6	1.9	1.3	3.3	.3	4.8	.5
3	10.0	5.2	.9	.3	3.4	.9	.0	3.4
5	24.1	7.0	.0	.0	3.9	.0	5.8	.0
10	7.2	.2	2.1	.2	8.5	.0	0.0	3.4
15	10.4	3.0	.9	1.1	8.0	.9	.9	.0
20	8.2	.6	.6	.0	5.3	.0	.0	.0
25	4.8	.0	.0	.0	.0	.0	2.9	3.0
30	9.4	.9	1.1	.0	3.2	.3	.0	1.7
35	8.1	.03	.0	.0	1.3	.2	.0	.0
40	1.2	1.1	.6	.0	1.9	.0	1.0	.0
45	2.5	.04	.0	.0	.9	.0	.0	.0

[1] Average of 3 trials in all cases except *P. triticina*, in which there were 1 and 2 trials, respectively.

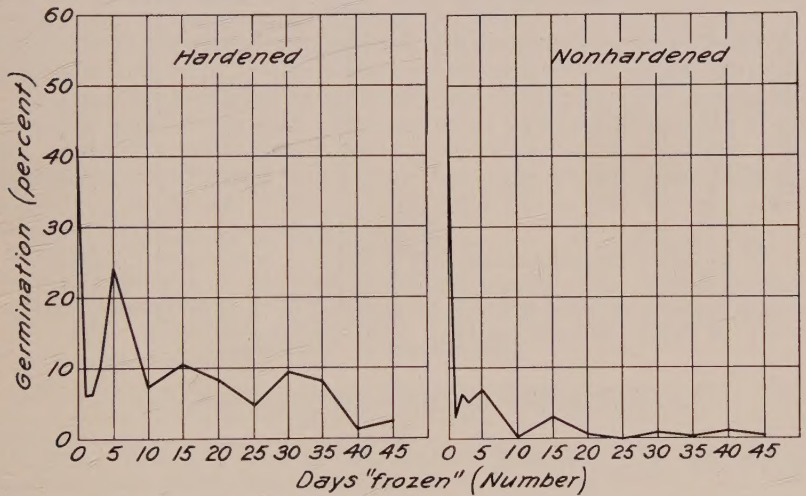

FIGURE 1.—Effect of hardening on resistance of urediospores of *Puccinia graminis tritici* form 15 to temperatures of −29° to −40° C.

EFFECT OF ALTERNATE FREEZING AND THAWING ON VIABILITY OF UREDIOSPORES

Stakman and Piemeisel (*24*) suggested that the reason urediospores of *Puccinia graminis tritici* do not survive the winter in Minnesota is that they are killed by "alternate freezing and thawing" in the spring. Lambert (*12*) also intimated that the reason urediospores survived the winter of 1923–24 at San Antonio and not at Dallas, Tex., was that alternate freezing and thawing occurred only 4 times at San Antonio and 20 times in the Dallas district.

Attempts were made to ascertain what effect temperatures alternating between −29° to −40° and +20° C. had on the viability of urediospores under dry conditions. Sets of spore-dusted slides were prepared, sufficient in number to permit germination tests at the end of 1, 2, 3, 6, 9, 12, and 15 days, respectively. The slides were kept alternately at the subzero temperature −29° to −40° for 24 hours and at 20° for 24 hours, and a control set was kept at −29° to −40°.

For the first 3 days of the experiment there was no significant difference in the survival of urediospores of *Puccinia graminis tritici* form 15 and *P. triticina* when exposed alternately to subzero and above-zero temperatures or to constant low temperatures (table 3). However, after 6 days' exposure to temperatures alternating between −29° to −40° and 20° C., spores of *P. triticina* were more severely

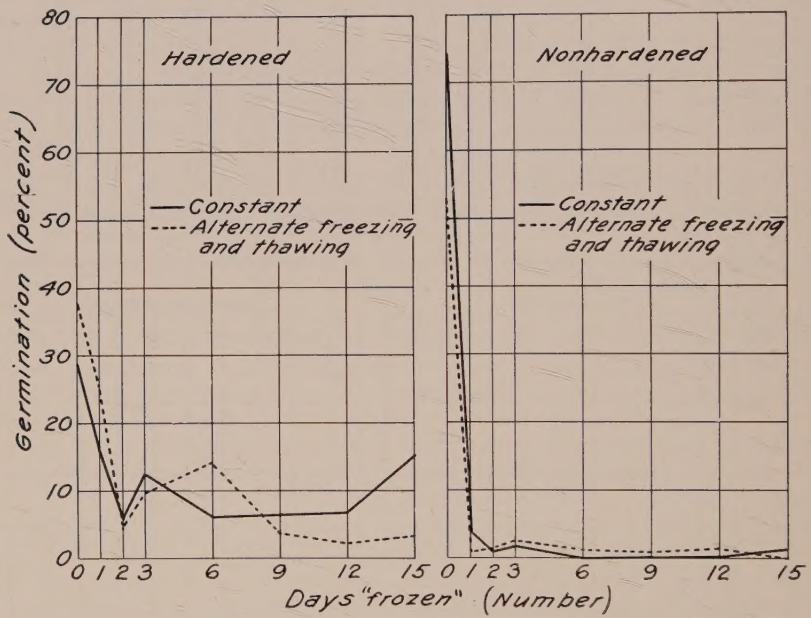

FIGURE 2.—Effect of alternating subzero and above-zero centigrade temperatures on the viability of hardened and nonhardened urediospores of *Puccinia graminis tritici* form 15.

injured than at a constant temperature of −29° to −40°. On the other hand, there appeared to be no consistent differential effect of constant and of alternating temperatures on urediospores of *P. graminis tritici*, except in the case of hardened spores after 9 days (fig. 2), when the spores withstood constant low temperature better than alternating temperatures. In all cases hardened urediospores withstood the low temperature better than nonhardened spores (fig. 2).

Of the spores of *Puccinia graminis tritici* exposed to alternating temperatures, more nonhardened than hardened spores were killed by the first exposure to a subzero temperature. After the marked diminution in percentage of germination during the first 2 days of exposure, there was no perceptible further diminution in hardened

spores until they had been exposed alternately to subzero and above-zero temperatures during a period of 9 days (fig. 2).

TABLE 3.—*Effect of alternate subzero and above-zero centigrade temperatures on viability of hardened and nonhardened urediospores of Puccinia graminis tritici and P. triticina*

Days at −29° to −40° C. alternating with 20° (number)	Percentage of germination [1]				Days at −29° to −40° C. (number)	Percentage of germination [1]			
	P. graminis tritici form 15		*P. triticina*			*P. graminis tritici* form 15		*P. triticina*	
	Hardened	Nonhardened	Hardened	Nonhardened		Hardened	Nonhardened	Hardened	Nonhardened
0	37.6	52.9	55.8	23.9	0	28.9	74.1	56.3	47.7
1	24.9	.7	42.6	3.6	1	16.0	3.8	22.4	10.9
2	4.4	1.3	9.4	2.6	2	5.9	.9	30.8	6.4
3	9.8	2.3	29.9	3.0	3	12.5	1.8	17.6	3.8
6	14.1	1.2	6.5	.4	6	6.1	.0	18.8	2.5
9	3.6	.8	7.8	.0	9	6.5	.3	32.9	11.0
12	2.1	1.3	9.1	.3	12	6.7	.3	27.7	6.6
15	3.1	.0	1.4	.1	15	15.2	1.4	22.5	1.8

[1] Average of 2 trials.

Effect of Temperature on Length of Incubation Period

Stakman and Lambert (*21, p. 374*) concluded that "* * * in the past, there has been a tendency for destructive epidemics to develop in warm growing seasons, and for cool seasons to be comparatively free from rust." Temperature undoubtedly influences the length of the incubation period of rust and therefore influences the number of uredial generations and the consequent increase of inoculum with each successive generation. Four generations during a warm growing season may furnish very large amounts of inoculum, but with only two generations during a cool growing season the increase in inoculum would be appreciably less.

Several investigators have reported results of experiments on the effect of temperature on the incubation period. According to Stakman and Levine (*22, p. 69*), uredia of *Puccinia graminis* may appear 6 or 7 days after inoculation at a temperature of 66.5° to 70° F. Their results show that—

> at low temperatures the development of the uredinia was retarded at the rate of 1 day for every 5 degrees of fall in temperature. Infection resulted at as low temperatures as the host could possibly stand. The spores were rather small, but the difference was not as great as in the case of high temperatures, with moderate temperature as a basis for comparison.

Mehta (*16*) found that uredia of *P. graminis* appeared 21 to 22 days after inoculation at a mean temperature of 40° F., in 14 to 15 days at 50.8°., in 11 to 12 days at 54.1°., and in 8 to 9 days at 66.5°. In 1923 Peltier (*17, p. 49*) concluded as follows regarding *P. graminis tritici* form 9:

> However, plants held at 5° for 9 weeks did not develop any rust until they were transferred to a higher temperature. On the other hand, the incubation period of the rust on plants placed in the 10°, 15°, 20°, 25°, and 30° cases was 18, 16, 11, 8, and 8 days respectively. * * * Plants inoculated at 5° C. and maintained at this temperature for 7 weeks developed no rust until they were transferred to a higher temperature.

The writer made experiments to determine the effect of different temperatures on infection and on subsequent development of the rust. The effect of temperature on the entrance and the establishment of the rust in the host was determined first. Three series of plants were inoculated and placed in incubation chambers for 48 hours at 0° to 1°, 10°, and 20° C., respectively. Then, to determine the effect of temperature on the subsequent development, one-third of each set was placed in a room at 20°, another third at 10°, and the remainder at 0° to 1°. All of the plants were kept constantly in a light of about 304 foot-candles.

Puccinia graminis tritici form 35 and *P. graminis avenae* form 2 caused no infection on plants kept in moist chambers for 48 hours at 0° to 1° C. and subsequently kept at the same or at higher temperatures (table 4). On the other hand, rye plants inoculated with *P. graminis secalis* form 7 and incubated similarly at 0° to 1° produced uredia at the end of 17 days at 10° and after 10 days at 20°. It is possible, of course, that the parasite may have gained entrance into the host after the latter had been placed in the warmer temperatures, but this seems unlikely.

TABLE 4.—*Time required for appearance of uredia of 3 varieties of Puccinia graminis at different temperatures under constant light*

Incubation temperature in—		Time required for first appearance of uredia of—[1]		
Moist chamber (48 hours)	Constant light room	*P. graminis tritici* form 35	*P. graminis secalis* form 7	*P. graminis avenae* form 2
° *C.*	° *C.*	*Days*	*Days*	*Days*
20	0–1	70	44	50
	10	14	13	14
	20	8	8	9
10	0–1	----------	54	----------
	10	16	16	17
	20	9	8	9
0–1	0–1	----------	----------	----------
	10	----------	17	----------
	20	----------	10	----------

[1] Each figure is the average of three trials.

At 10° and 20° C. the urediospores of the above-mentioned forms of *Puccinia graminis* germinate and cause infection readily. A temperature of about 20° C. seems to be the optimum for the entrance and establishment of the pathogene.

The three varieties of *Puccinia graminis* mentioned above can develop and produce uredia at 0° to 1°, 10°, and 20° C., if given opportunity to gain entrance and to become established in the host at an appropriate temperature (table 4). However, more time was required for urediospores to appear at the lower temperatures. At 0° to 1°, uredia of *P. graminis tritici* form 35 did not appear until 70 days after inoculation. In another experiment, *P. graminis tritici* form 15 produced urediospores normally within 10 or 11 days at 20° and within 18 days at 10°, whereas at 0° to 1° uredia failed to appear within 80 days. This indicates that physiologic forms of *P. graminis tritici* differ in their ability to develop at temperatures just above freezing.

The results suggest that in nature the mycelium may remain dormant in infected plants during the winter if the host tissues remain alive. If this happens, urediospores may be produced as soon as the weather becomes warm; and, if there are susceptible hosts nearby, infection occurs. Several weeks of cool weather then may follow infection and the mycelium may lie dormant until the temperature again rises. Then the uredia may "miraculously" appear, furnishing early inoculum to infect young grain plants and start an early epidemic.

Influence of Temperature on Development of Mycelium within Host Tissue

Some field observations have been made on the survival of stem rust mycelium from one season to another. Lambert (*12*) reports that at Dallas, Tex., the host leaves bearing old pustules were killed by alternate freezing and thawing. Mehta (*16*) states the following conclusions from his observations:

> It is clear that the mycelium of black rust can survive milder weather and but for the absence of the source of infection, it might appear on the cereal crops in the earlier part of spring, if weather conditions are favorable.

Obviously, the host tissue must survive the winter if the rust mycelium is to survive. The question then arises as to whether the mycelium of *Puccinia graminis* can withstand as low temperatures as the host. The following experiments accordingly were made.

Three sets of wheat differentials were inoculated with urediospores of *Puccinia graminis tritici* form 15, incubated in a moist chamber at 20° C. for 48 hours, and then placed in the 0°, 10°, and 20° chambers, respectively. This experiment was repeated three times. Uredia developed on plants placed at 10° and 20°, but at the end of 60 days none had developed at 0°. Plants in the 0° room were removed to the greenhouse at the end of 40, 50, 60, 70, 80, and 90 days, respectively. On plants kept at 0° to 1° for 80 days and then transferred to 20°, uredia appeared within 8 days after the transfer, but none appeared on plants kept at 0° to 1° for 90 days.

The mycelium of *Puccinia graminis avenae* form 2 lived for 80 days at 0° to 1° C. without producing uredia, but they appeared 4 days after the infected plants were removed to the greenhouse.

In another experiment, seedling wheat plants, inoculated with *Puccinia graminis tritici* form 15 and kept in moist chambers at 20° C. for 48 hours, were placed in the 10° room under constant light and allowed to remain there until flecks developed. Then they were removed to the 0° to 1° room, and 32 days later uredia appeared.

To determine whether the mycelium of *Puccinia graminis* can withstand as low temperatures as the host plant, seedling plants of hardy winter varieties were inoculated, kept in moist chambers for 48 hours, and then placed in the greenhouse. When flecks appeared, the plants were placed in the 0° C. chamber for at least 10 days to allow them to harden, after which different sets were kept at −12°, −10°, −7°, and −5°, respectively, for 24 hours. The same procedure was followed with plants in tillering and boot stages. After freezing, the plants again were placed at 0° for 24 hours to allow them to "thaw out" gradually. They then were removed to the greenhouse and were watched for the appearance of stem rust. Each of the experiments was repeated several times.

Uredia of *Puccinia graminis tritici* form 35 appeared on Minhardi wheat in the greenhouse about 3 days after having been subjected to −5° C. for 24 hours. In a later experiment, uredia of *Puccinia graminis tritici* form 15 appeared on Kanred and Minturki wheats in 3 and 6 days, respectively, after having been subjected to −10° for 24 hours. In this experiment low temperature seemed to affect the organism more in plants of Minturki wheat than in those of Kanred wheat, as it took the uredia 3 days longer to appear. In subsequent tests at −13° the infected leaves were killed. Uredia of *Puccinia graminis secalis* form 7 appeared on plants of Petkus rye that had been exposed to −7° for 24 hours.

It is clear that at low temperatures, the rust mycelium can remain dormant for long periods in the host, and it apparently can withstand as low a temperature (−10° C.) as the host plants. However, lower temperatures must be tried to demonstrate the latter point conclusively.

Effect of Temperature on Infection Type

Stakman and Levine (*22*, *23*) and Levine (*13*, *14*) have shown that environmental conditions unfavorable for the host are unfavorable also for the parasite, affecting the virulence and spore size of the latter. Waterhouse (*25*, *p. 676*) recently reached the following conclusion from his experiments:

> In the case of *Puccinia graminis tritici* there was change from complete susceptibility in the hotter months to complete resistance in the cooler part of the year. The reactions on several of the differential hosts showed this change. Some physiologic forms show much more marked alterations of this nature than do others. Changes from summer to winter conditions alter the reactions shown by *Puccinia graminis avenae* on "Joanette" from complete susceptibility to complete resistance.

The writer made a study of the effect of temperature on the infection type. Even at 0° C. some rust appeared on plants that had previously been inoculated and kept for 48 hours in a moist chamber at 20°, but the uredia were very small. There was no significant difference in infection types at 10° and 20°, the only noticeable difference being a higher proportion of type 3 to type 4 at 10 °. However, not only were much smaller pustules produced by several physiologic forms at 0° to 1° than at 20°, but the areas surrounding the pustules were strongly etiolated. The uredia thus developed resembled type 1 as long as the plants remained at 0° to 1°, but they changed to type 3 or type 4 when the plants were placed at 20°. For example, Little Club wheat at 20° is moderately to completely susceptible to *Puccinia graminis tritici* form 35, infection types 3 and 4 ordinarily being produced; but at 0° to 1° pustules approaching type 1 were produced 70 days after inoculation in 2 out of 3 trials (fig. 3). In a later experiment, seedlings of Little Club, inoculated on February 27 with spores of *Puccinia graminis tritici* form 35, were placed at 0° to 1° on March 6. Thirty days later uredia similar to type 1 appeared.

A set of differential varieties of wheat, inoculated with *Puccinia graminis tritici* form 15 and kept at 10° C. until flecks appeared, was placed in the 0° to 1° chamber. After 32 days, pustules of "type 1",[5] instead of the characteristic type 3 or 4, appeared on 11 of the differential hosts (fig. 4). The first leaves of plants on which the apparent

[5] Small pustules approaching type 1 are designated "type 1."

type 1 pustules were produced at 0° to 1° were cut off, and new leaves were allowed to develop at 20°. They then were inoculated with spores of form 15, and this time the plants were allowed to remain at 20°. Normal vigorous pustules of types 3 and 4 resulted (fig. 4).

FIGURE 3.—"Type 1" uredia produced on Little Club wheat by *Puccinia graminis tritici* form 35 at 0° to 1° C. This form normally produces uredia of types 3 and 4 on Little Club wheat at 20°.

Inoculation with spores from the type 1 pustules, produced at 0° to 1° on Vernal emmer, resulted in the development of type 4 uredia when cultured at 20°. Type 1 pustules of form 15, previously formed at 0° to 1°, developed into type 3 after the plants were removed to

20°. *Puccinia graminis secalis* form 7 and *Puccinia graminis avenae* form 5 produced normal uredia even at 0° to 1°. The type 1 uredia, therefore, represented merely the lower limit of development of rust under abnormal conditions.

Difference in Ability of Different Physiologic Forms to Develop at 0° to 1° C.

Physiologic forms of *Puccinia graminis* seem to vary in their ability to develop at low temperatures. This is shown by the following experiment. On February 3, 1930, 10 pots of Little Club wheat were

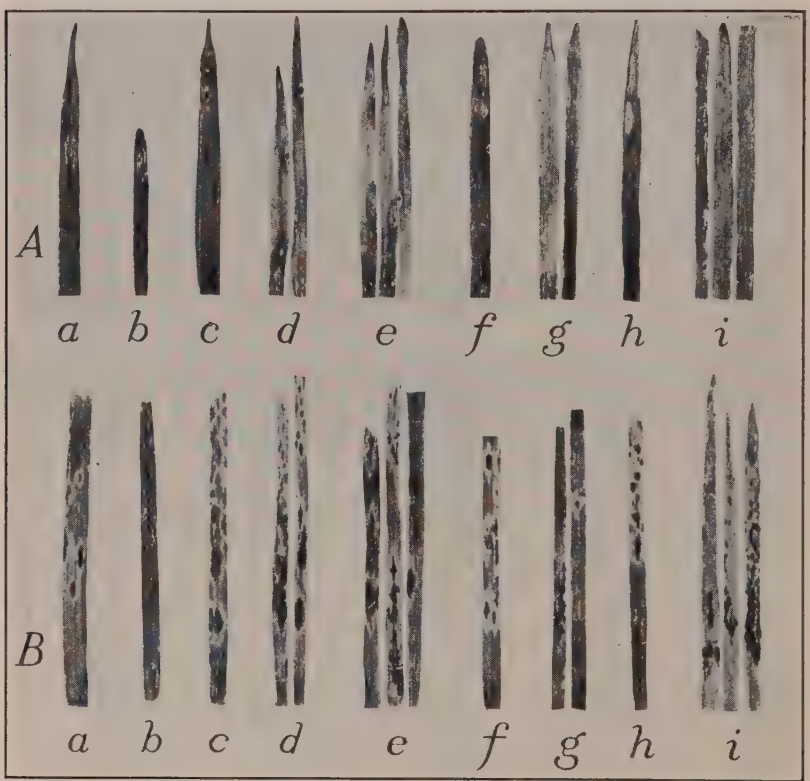

Figure 4.—*A*, Minute uredia (type 1) produced by *Puccinia graminis tritici* form 15 on varieties of *Triticum* spp. at 0° to 1° C.; *B*, normal development of rust at about 20° on secondary leaves of plants shown in *A*. *a*, Marquis; *b*, Kanred; *c*, Arnautka; *d*, Mindum; *e*, Spelmar; *f*, Kubanka; *g*, Acme; *h*, einkorn; *i*, Vernal emmer.

inoculated with *P. graminis tritici* form 15 and 10 with form 36. The plants were kept in moist chambers under the same conditions for 48 hours and then placed in the same greenhouse for 4 days. At the end of this period (Feb. 9) they were placed at 0° to 1° C. under constant light. Uredia of form 36 appeared on February 21, and only one uredium of form 15 had appeared by March 18, while form 36 had sporulated and produced numerous normal uredia (fig. 5). Pustules of *P. graminis tritici* form 15, however, appeared shortly after the plants again were placed in the greenhouse, showing that the mycelium

had remained alive at 0° to 1°, although there had been no appreciable development.

Two forms of *Puccinia graminis avenae* (forms 2 and 5) seemed also to develop at different rates at 0° to 1° C. For example, on February 3, 1930, several pots each of Victory oats were inoculated with these forms and kept in moist chambers under the same conditions. After 48 hours they were removed to the same greenhouse and kept 4 days. On February 9 they were placed at 0° to 1° under constant light. Uredia of form 5 appeared on February 22, while uredia of form 2 did not appear until March 3.

Evidently there are differences in the ability of physiologic forms of *Puccinia graminis tritici* and *P. graminis avenae* to develop at 0° to 1° C., whereas at 20° there are no apparent differences.

Form 7 of *Puccinia graminis secalis* developed well at 0° to 1° C., almost as well as at 10° and 20°, perhaps accounting for the prevalence of rye rust in the later fall in northern regions.

Low temperatures seem to stimulate the development of telia, because this stage of *Puccinia graminis secalis* form 7 formed abundantly

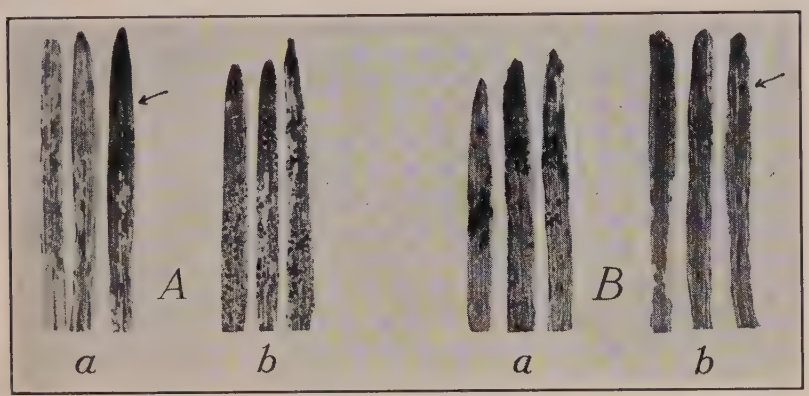

FIGURE 5.—Variation in ability of physiologic forms of *Puccinia graminis* to produce uredia at 0° to 1° C. *A*, Leaves of Little Club wheat seedlings infected with *P. graminis tritici: a*, Form 15; *b*, form 36. *B*, Leaves of Victory oat seedlings infected with *P. graminis avenae: a*, Form 5; *b*, form 2.

at 0° to 1° C. *P. graminis tritici* form 15 and form 36 produced abundant telia after the infected plants had been transferred from 0° to 1° to a warm greenhouse, where telia do not ordinarily form in abundance.

EFFECT OF LIGHT INTENSITY

The effect of light on certain rusts has been studied by several investigators. Fromme (*7, p. 519*) found that "Total light exclusion either early or late in the incubation period checks the development of *P. coronifera* and results in an almost complete cessation of growth." Mains (*15*) studied the nutritional phase and arrived at the following conclusions: "When the host is deprived of carbohydrate, *P. coronata* and *P. sorghi* require light in order to develop. However, starvation will not bring about immunity." Stakman and Piemeisel (*24*) pointed out that a good deal of sunlight is necessary for the best development of stem rust. They state (*24, p. 487*):

> During periods of cloudy weather, however, the incubation period may be lengthened a week or more, and the rust does not develop so abundantly as

during bright weather. * * * The shaded plants were more weakly infected than the others. Partially etiolated plants were infected with difficulty and the rust developed very weakly on them.

Peltier (*18*) concluded that light is not a factor in the initial infection of wheat by stem rust, but it is essential for subsequent development. Gassner (*8*) also pointed out that light governs rust development in proportion to the effect it has on the metabolism of the host plant and that high light intensities tend to shorten the incubation period.

The writer made experiments with *Puccinia graminis* to determine the effect of different light intensities, at uniform temperature and humidity, on length of incubation period, type of infection, and morphology of urediospores.

Three sets of differential varieties of wheat and oats, after being inoculated and kept in moist chambers for 48 hours under uniform conditions, were placed in the 20° C. chamber under light intensities of 394, 301, and 162 foot-candles, respectively.

Under all degrees of light used the plants were much lighter in color than those grown under natural light in the greenhouse. There was slight etiolation under 394 and 301 foot-candles and considerable etiolation under 162 foot-candles. In general, oats did not thrive so well as wheat in artificial light, and plants of both cereals became very weak and etiolated after about 3 weeks. Moreover, root rot seriously hindered the growth of the plants, greater damage resulting under the light intensity of 162 foot-candles than under the higher intensities. Marquis wheat was least affected, while the durum varieties Arnautka and Spelmar were heavily infected at low light intensities. Rye thrived under artificial light for a period of about 3 weeks, after which the plants died rapidly.

Effect of Different Light Intensities on Length of Incubation Period

High light intensities shortened the incubation period appreciably. Under an intensity of 394 foot-candles, uredia of *Puccinia graminis tritici* form 15 appeared in approximately 9 days, while under the lower intensities of 301 and 162 foot-candles 10 to 11 days were required. There were no consistent differences in incubation periods in different varieties of *Triticum* spp. Uredia of *P. graminis avenae* form 2 appeared in 8 to 9 days under 394 foot-candles, while 9 to 12 days were required for their appearance under the lower light intensities.

Effect of Different Light Intensities on Type of Uredia Produced

A study was made of the effect of different light intensities on the infection types produced by *Puccinia graminis avenae* form 2 on 4 differential oat varieties and by *P. graminis tritici* form 15 on 11 differential wheat varieties. In addition, a comparative study was made of the effect of the same light intensities on the infection types produced by three varieties of *P. graminis*, namely, *P. graminis tritici* form 35 on Little Club wheat, *P. graminis secalis* form 7 on Petkus rye, and *P. graminis avenae* form 2 on Victory oats.

The results indicate that the different degrees of light used caused no significant or permanent changes in type of infection, although deficient light causes pustules to be considerably smaller.

From these experiments it may be concluded that under low light intensities the normal development of stem rust pustules is inhibited, resulting in the production of smaller pustules, but there is no indication of a significant or permanent change in type of infection; there is no indication that certain hosts that are susceptible under intense light are rendered resistant under low light.

Effect of Different Light Intensities on Size and Shape of Urediospores

Urediospores produced under the various light intensities were measured and compared with those produced under sunlight at 20° C. Some similar studies had been made by Levine (*13*), who found that urediospores produced in deficient light were shorter than normal.

Table 5.—*Variants and constants for dimensions of urediospores of Puccinia graminis tritici form 15 grown under different light and temperature conditions in the greenhouse*

LENGTH

Cultural conditions		Range of variability	Constants		
Temperature (°C.)	Light		Mean	Standard deviation	Coefficient of variability
	Foot-candles	μ	μ		
10	334	27.83–46.46	35.50±0.19	4.01±0.14	11.29±0.39
20	394	26.91–43.47	35.19± .16	3.39± .11	9.63± .32
	301	25.53–45.08	36.13± .17	3.54± .12	9.80± .33
	162	27.60–42.78	34.41± .16	3.42± .12	9.94± .34
	(1)	25.76–44.85	34.54± .17	3.54± .12	10.24± .35

WIDTH

10	334	17.94–27.14	22.03±0.07	1.56±0.05	7.09±0.24
20	394	18.86–27.14	22.11± .07	1.39± .05	6.27± .21
	301	19.55–27.60	21.90± .07	1.55± .11	7.07± .24
	162	17.25–25.76	21.77± .08	1.70± .06	7.80± .26
	(1)	19.32–27.60	23.03± .07	1.52± .05	6.59± .22

RATIO OF LENGTH TO WIDTH

		Correlation coefficient			
10	334	−0.107±0.047	1.637±0.011	0.23±0.01	14.29±0.48
20	394	− .239± .045	1.621± .010	.21± .01	12.79± .43
	301	− .255± .045	1.678± .011	.22± .01	13.26± .45
	162	− .197± .046	1.621± .011	.22± .01	13.80± .47
	(1)	− .242± .045	1.427± .010	.20± .01	14.20± .48

[1] Sunlight.

In this study the length and width of 200 spores from each of the different cultural conditions were measured at random on a slide. Because the differences were not great, it was necessary to employ statistical methods to make comparisons (tables 5 and 6). There was negative correlation between length and width of spores in all lots measured. Spores produced under 301 foot-candles were significantly longer but not significantly wider than those under 162 foot-candles, and they were significantly longer but narrower than those produced in sunlight at 20° C., and as a result of this differed

from the latter also in shape, i. e., in ratio of length to width. Spores produced under 394 foot-candles were narrower, though not significantly longer, and quite different in shape from those produced in sunlight at 20°. Those produced under 162 foot-candles were narrower than spores produced in sunlight at 20° and differed from them in shape. Spores produced under 334 foot-candles at 10° did not differ significantly from those produced under 394 foot-candles at 20°.

TABLE 6.—*Differences between means of dimensions of urediospores of Puccinia graminis tritici form 15 cultured under different light and temperature conditions*

Cultural conditions compared	Length		Width		Ratio of length to width	
	Difference in means	Difference divided by P. E.	Difference in means	Difference divided by P. E.	Difference in means	Difference divided by P. E.
	μ		μ			
334 foot-candles at 10° with 301 foot-candles at 20°	0.63	2.46	0.13	1.31	0.041	2.66
394 foot-candles at 20° with 301 foot-candles at 20°	.94	4.01	.21	2.12	.057	3.93
394 foot-candles at 20° with 162 foot-candles at 20°	.78	3.40	.34	4.23	.000	.00
301 foot-candles at 20° with 162 foot-candles at 20°	1.72	7.35	.13	3.20	.057	3.73
394 foot-candles at 20° with sunlight at approximately 20°	.65	2.78	.92	9.29	.194	13.96
301 foot-candles at 20° with sunlight at approximately 20°	1.59	6.66	1.13	11.41	.251	17.43
162 foot-candles at 20° with sunlight at approximately 20°	.13	.56	1.26	11.85	.194	13.20

DISCUSSION AND CONCLUSIONS

The results obtained show that the death of urediospores in the winter probably is not always due to low temperatures as such. This is shown by the fact that some urediospores of the *tritici* and *phleipratensis* varieties of *Puccinia graminis* remained viable after having been exposed for 45 days to temperatures ranging from −29° to −40° C. It is true that a large percentage of urediospores usually are killed within the first 24 hours after being exposed to the low temperature, but most of those that survive retain their viability for a long time.

Hardened urediospores are more likely to survive than those not hardened. Spores produced under normal conditions were hardened at 0° C. for 10 days or longer, and their viability was compared with that of similarly produced, nonhardened spores. The hardened spores were more resistant to cold than the nonhardened. It seems probable, therefore, that spores formed at low temperatures in the fall may be more cold-resistant than those formed at higher temperatures.

Not only are some urediospores able to withstand continuous low temperatures, but a few may withstand fluctuating temperature in a surprising manner, especially when dry. It seems quite likely, therefore, that the death of urediospores in nature during the northern winters is due neither to low temperature nor to alternating low and moderate temperatures when the spores remain relatively dry, but rather to alternate freezing and thawing during wet weather.

Uredial mycelium apparently can withstand temperatures as low as those endured by the host plants. For example, mycelium remained viable within infected wheat plants kept for 24 hours at −10° C. When the plants were again placed at a temperature of approximately 20°, rust pustules developed within a few days The mycelium within infected rye plants withstood a temperature of −7° for 24 hours and subsequently produced pustules. In nature, therefore, the mycelium probably can survive in any plant tissues that remain alive during periods of cold weather. The experiments indicate also that this mycelium can subsequently produce urediospores with the advent of favorable temperatures. This is shown by the fact that both wheat and oats infected with rust were kept for 80 days at a temperature of 0°, during which time no uredia appeared; when the plants were transferred to the greenhouse, however, uredia appeared in 8 days.

Temperature also has a profound effect on the development of rust in normal plants after infection once has taken place. For example, it required approximately a week longer for uredia to appear at 10° C. than at 20°. According to this, for every drop of 1° in temperature the incubation period would be lengthened about two-thirds of a day. This probably is not an absolute rule, as there is considerable variation in the effect of temperature on the incubation period. The generalization would be approximately true only for a range of temperatures in which the rust could develop fairly well. The fact, however, is very significant, because, in the spring wheat area of the United States, there is a relatively short time in which rust develops. Usually it is only about 45 days. At high temperatures, therefore, about 6 crops of urediospores would be produced during the season, while at low temperatures only 2 or at most 3 successive crops would be produced, and the amount of inoculum and subsequent development of rust would be greatly reduced. Field observations indicate that relatively slight differences in temperature during the critical period for the development of rust, other things being equal, determine the severity of a rust epidemic. In many years, there is a very close race between grain crops and rust. The greatest damage usually occurs just before harvest; and in many seasons the crops escape severe damage if the rust parasite is unable to produce a large amount of inoculum at that time.

Excessively low temperatures may greatly lengthen the incubation period. For example, in some experiments it required 70 days for form 35 of *Puccinia graminis tritici* to produce uredia at 0° to 1° C., and some physiologic forms seem to be entirely unable to produce uredia at so low a temperature. It is likely that the effect of temperature may vary with the particular physiologic forms concerned in the development of rust in a given locality or in a given season.

Temperature is important not only in governing the length of the incubation period and the consequent amount of inoculum produced in a given season but also in determining the size of uredia and consequently the number of spores on each infected plant. This is clearly shown by the fact that at 0° to 1° C. *Puccinia graminis tritici* forms 15 and 35 produced pustules resembling type 1 pustules, which are extremely small, whereas these same forms produced type 4 pustules on the same variety at higher temperatures. The small pustules, however, may enlarge if the temperature rises. Tempera-

ture therefore determines the amount of inoculum in two ways: (1) by determining the number of urediospore generations in a given season and (2) by determining the number of spores per pustule.

The ability of certain physiologic forms of *Puccinia graminis* to develop at low temperatures may be important from the standpoint of regional epidemics of stem rust in the spring wheat area. If inoculum of these forms is present in this area early in the grain-growing season, stem rust can increase regardless of cool weather. This complicates the stem rust problem further, because low temperature retards the development of many forms of *Puccinia graminis tritici* sufficiently to inhibit severe infection.

As temperature extremes seem to induce early teliospore formation, teliospores are likely to be produced in nature when it is either abnormally hot or abnormally cool and when the potential danger of rust is consequently reduced. However, the effect of moisture must not be overlooked in this connection.

Light, of course, affects rust development. However, in the experiments made by the writer, the differences in light intensity were not sufficiently great to change the host reaction from susceptible to resistant or vice versa. There were fluctuations, but a type 4 infection was never reduced to a type 1 or a type 2 by withholding light. It seems unlikely that in nature there would be sufficient variation in total light during any given season to affect appreciably the development of rust.

The experiments indicate that temperature is the most important factor governing the development of rust in a susceptible host, assuming, of course, that there is sufficient moisture for infection and enough light for normal growth of plants. The stem rust pathogene seems to be well equipped to withstand vicissitudes, for it is able to persist at a very low temperature. However, its development to epidemic proportions is conditioned by temperature, as pointed out by Stakman and Lambert (*21*).

SUMMARY

Hardened urediospores of *Puccinia graminis* withstood subzero centigrade temperatures better than nonhardened urediospores. Hardened urediospores of wheat stem rust (*Puccinia graminis tritici*) and of timothy stem rust (*Puccinia graminis phlei-pratensis*) were able to withstand subzero temperatures for at least 45 days and hardened urediospores of oat stem rust (*Puccinia graminis avenae*) for 40 days. A few nonhardened urediospores of wheat stem rust withstood 45 days of subzero temperature; timothy stem rust, 35 days; and oat stem rust, 15 days.

Temperatures alternating above and below 0° C. had no more killing effect on nonhardened urediospores of *Puccinia graminis tritici* form 15 than did constant subzero temperatures. On the other hand, alternating temperatures killed more hardened spores after 9 days than did constant subzero temperatures.

The incubation period of stem rust is lengthened by low temperature. It took approximately a week longer for uredia to appear on plants kept at 10° C. than at 20°. Uredia developed at 0° to 1°, but much more time was required. *Puccinia graminis tritici* form 35 produced uredia in about 70 days at 0° to 1°. When the infected

plants were placed at 0° to 1° 48 hours after inoculation, form 15 produced no uredia during 80 days' exposure at this low temperature.

The mycelium of at least some physiologic forms of stem rust can withstand as low temperatures as the host. This was true of *Puccinia graminis tritici* form 35 and *Puccinia graminis secalis* form 7.

Except under extreme conditions, temperature has little effect on the type of uredia produced. At 10° C. smaller pustules were produced than at 20°. A temperature of 0° to 1° effected an apparent change of infection type of *Puccinia graminis tritici* forms 15 and 35. An infection type similar to type 1 was produced at 0° to 1° in place of type 3 or 4, normally produced at 20°. However, after the infected grain plants were taken from 0° to 1° and placed in the greenhouse at 20°, the type 1 infection developed into type 3. Therefore this temporary change at 0° to 1° was due to low temperature.

Physiologic forms of *Puccinia graminis tritici* and *Puccinia graminis avenae* differed in ability to produce uredia at low temperatures. At 0° to 1° C., *Puccinia graminis tritici* form 36 produced normal uredia readily; forms 15 and 35 produced only a few minute pustules similar to a type 1 infection; *Puccinia graminis avenae* form 2 did not produce uredia as readily as form 5. *Puccinia graminis secalis* form 7 also produced normal uredia at 0° to 1°.

A temperature of 0° to 1° C. stimulated the production of telia.

Different light intensities had no significant effect on the infection type produced, but low light intensities delayed the appearance of uredia. It took 1 to 2 days longer for uredia of *Puccinia graminis tritici* form 15 and *Puccinia graminis avenae* form 2 to appear under light intensities of 301 or 162 foot-candles than under a light intensity of 394 foot-candles.

Light intensities and quality of light seemed to affect the size and shape of urediospores. Urediospores of *Puccinia graminis tritici* form 15, formed under a light intensity of 301 foot-candles at 20° C., were significantly longer than those produced under other light conditions. Urediospores produced under artificial light were significantly longer and narrower than those produced in the greenhouse.

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